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Structure, function and evolution of DnaJ: conservation and adaptation of chaperone function

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INTRODUCTION

It is a general feature of molecular chaperones that they are highly conserved throughout evolution. Prokaryotic and eukaryotic Hsp70 proteins, for example, are over 50% identical at the amino acid level. The DnaJ family of molecular chaperones, however, share this conservation only within a single 70 amino acid domain called the J domain, and outside of this region the family is greatly divergent. Such diversification probably contributes to the varied actions of molecular chaperones in protein dynamics, from folding of nascent polypeptide chains to clathrin uncoating. DnaJ proteins function as part of the Hsp70 chaperone machine, since the J domain is known to stimulate the ATPase activity of Hsp70 proteins. This review will concentrate on recent progress in DnaJ research. Readers who are new to the field are encouraged to read previous reviews on the family by Caplan et al 1993; Silver and Way 1993, and Cyr et al 1994.

DNAJ FAMILY NOMENCLATURE

One of the common problems for newcomers to DnaJ family research is to realize that DnaJ-like proteins are not all subtle variations on a theme, in fact they can be extremely diverse. Much of our knowledge of DnaJ family function comes from the *Escherichia coli* protein DnaJ. *E. coli* DnaJ has four canonical domains and it would be a mistake to assume that other DnaJ-like proteins such as Hsp40 (Hdj1) and Sec63 are merely higher organism analogues of DnaJ, as they have very different domain structures. In an attempt to clarify this, we propose the following nomenclature for the DnaJ family based upon the degree of domain conservation with E. coli DnaJ; Type I – full domain conservation with DnaJ; Type II – N-terminal J and G/F domains; Type III – J domain only (see Fig. 1 and Table for classification of known family members).

OVERVIEW OF *E. COLI* DNAJ PROTEIN STRUCTURE

E. coli DnaJ can be subdivided into four domains on the basis of its primary amino acid sequence. At the N-terminus is the J domain , a 70 amino acid α-helical region that

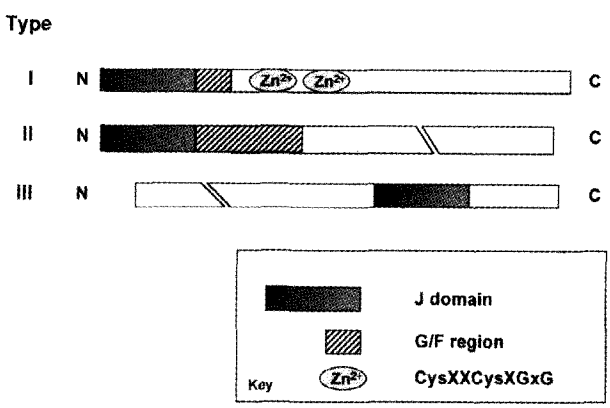

Fig. 1 Schematic representation of DnaJ family sub-types. Type I DnaJ proteins have full domain conservation with *Escherichia coli* DnaJ, type II have a J domain and a G/F domain (generally at the N-terminus) and type III proteins have only a J domain anywhere in the protein.

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Table DnaJ family members by type (Type I – all 4 *Escherichia coli* DnaJ domains; Type II – J domain and G/F domain; Type III – J domain alone). For full referencing see Genbank links to nucleotide sequences.

	Type I	Type II	Type III
Eubacteria	DnaJ	CbpA NolC	DjlA
Archaea	DnaJ		
Virus			T antigen
Yeast	YDJ1	SIS1	Sec63
	MDJ1	zuotin	JEM1
	SCJ1	CAJ1	YJL162c
	XDJ1	HLJ1	YNL227c
		YIR004w	YFR041c
		YJR097w	
Plant	ANJ1*	D3	
	ATJ1		
Animals #	HDJ2	HSJ1a & b	p58IPK
	Tid56	Hsp40(Hdj1)	MTJ1
			auxilin
			csp
			MIDA1

* Plant homologue of YDJ1–identified in many plant species.
# Not including Expressed Sequence Tags (ESTs).

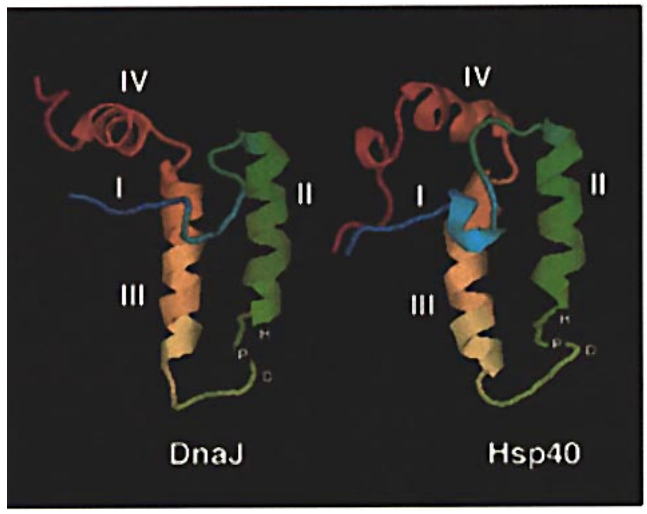

Fig. 2 Structure of the J domain. Comparison of the structures of the J domains from DnaJ (Pellecchia et al 1996) and Hsp40(Hdj1) (Qian et al 1996). The structures were downloaded from PDB (Abola et al 1987; Bernstein et al 1977) and viewed in RasMol. The structures are color coded, blue at the N-termini to red at the C-termini and helices numbered I to IV. The highly conserved HPD motif in the loop region is highlighted. Note the close similarity between the structures of the E. coli and human J domains, especially in the region of the anti-parallel α-helices. However, there are subtle differences between the two structures, particularly in the loop region and around the important HPD motif that may reflect the flexibility of this region. It is highly likely that all J domains will have this basic structure, but subtle differences in the loop region and projecting side groups from the helices may confer some specificity of interaction with Hsp70 sub-types.

interacts with Hsp70 and stimulates its ATPase activity. This is separated from the rest of the molecule by a 30–40 amino acid glycine/phenylalanine rich (over 40% glycine and 15% phenylalanine) region (G/F) that appears to be a flexible linker. Interestingly, in type II DnaJ proteins the G/F region tends to be longer, commonly 70–100 amino acids, with a more variable glycine content than type I G/F domains. In type I DnaJ proteins, distal to the G/F region is the cysteine rich domain that is organized into tertiary structure with the help of two Zn^{2+} atoms per molecule (Banecki et al 1996; Szabo et al 1996). Together, these three domains account for only half of the DnaJ molecule, the C terminal half is less well conserved among different J proteins and consequently is poorly understood. The following is a detailed account of the structure of these domains and how they relate to DnaJ family function.

Structure/function of the J domain and the G/F region

The presence of a J domain defines a protein as a member of the DnaJ family. It is present at the N-terminus of E. coli DnaJ, although it is found elsewhere in different eukaryotic DnaJ proteins (e.g. between membrane spanning domains in Sec63 and at the C-terminus of auxilin). The solution structure of the E. coli J domain has been solved (Fig. 2; Szyperski et al 1994; Hill et al 1995; Pellechia et al 1996). It is stable (melting temperature 75°C) and monomeric when expressed independently of other DnaJ domains in E. coli. The structure consists of 4 α-helices with no β-pleated structure. Helices II and III are anti-parallel amphipathic helices which are stabilized by a hydrophobic core. The short helices I and IV, situated at the N- and C-terminal ends of the J domain, respectively, appear to further stabilize the interaction between the anti-parallel helices.

The NMR solution structure of Hsp40(Hdj1) has also been solved (Fig. 2; Qian et al 1996) and shows that the primary sequence similarity between different J domains (approximately 50% between E. coli DnaJ and Hsp40) results in a very similar tertiary structure. Highly conserved residues in the J domain stabilize the anti-parallel coiled coil by inter-chain hydrophobic interactions. Less conserved residues appear to be on the outside of the central helical domain and may represent a mechanism for specificity in the regulation of different Hsp70 subtypes (see below).

The fundamental J domain structure is an exposed finger comprised of two tightly packed anti-parallel helices (II and III). The finger tip is a loop between helices II and III that contains the functionally significant and obligate HPD motif, found in all DnaJ-like proteins. The side chains of this tripeptide motif extend out from the core of the structure perhaps facilitating the interaction with Hsp70.

Genetic and biochemical studies have demonstrated that the J domain is the region of DnaJ that interacts with

Hsp70 and stimulates Hsp70 ATP hydrolysis. In particular, the HPD motif appears to be essential for this role since mutation in these amino acids, lead to a loss of function (Feldheim et al 1992; Wall et al 1994; Dey et al 1996; Tsai and Douglas 1996).

Recent studies have suggested that the J domain is necessary but not sufficient for interaction of E. coli DnaJ with Hsp70. Wall et al (1994) demonstrated that the first 108 amino acids of DnaJ, which includes both J domain and G/F regions, could enhance the ATPase activity of Hsp70(DnaK) and function in lambda replication, albeit with reduced efficiency compared with the wild-type protein. However, smaller polypeptide fragments that included only the J domain failed to show these effects, indicating that the G/F region was important for the J domain interaction with Hsp70(DnaK) (Karzai and McMacken 1996; Szabo et al 1996).

The role of the G/F region in stimulating J domain function also appears to depend on the peptide binding state of Hsp70(DnaK), since in the presence of bound peptide Hsp70(DnaK) ATPase activity can be stimulated by the J domain without the G/F region (Karzai and McMacken 1996). This suggests that the G/F region itself may contact with Hsp70(DnaK) thereby facilitating the J domain interaction, a contact that can be mimicked by peptides binding to the Hsp70(DnaK) peptide binding domain. In addition, a DnaJ mutant that had the G/F domain deleted bound substrate normally but failed to stimulate formation of a ternary complex with Hsp70(DnaK) (Wall et al 1995). These observations suggest that there may be a critical difference between type III DnaJ proteins, that do not possess the G/F domain, and type I and II proteins that do. It is possible that type III DnaJ proteins can only interact with Hsp70 when Hsp70 is already binding substrate, in contrast to type I and II proteins that can present substrate to Hsp70. However, studies of type III DnaJ proteins suggest that this may not be the case. For example, csp can enhance Hsp70(hsc70) ATP hydrolysis in a substrate independent manner (Braun et al 1996; Chamberlain and Burgoyne 1997) similar to type I (Liberek et al 1991; Cyr et al 1992) and type II proteins (Cheetham et al 1994), and another type III protein, auxilin, can stimulate Hsp70(hsc70) ATP hydrolysis (Jiang et al 1997) and facilitate the correct positioning of Hsp70(hsc70) on clathrin baskets (Ungewickell et al 1995). Another possibility is that type III DnaJ proteins have a very limited substrate specificity such that interaction in a binary complex would perfectly position the J domain for interaction with Hsp70. In this case, type I and type II DnaJ proteins would have a more relaxed substrate specificity with the G/F domain helping to correctly position the J domain for interaction with Hsp70.

So, although it is still not clear how the G/F domain modulates the interaction of the J domain with Hsp70, it appears that type III DnaJ proteins can also interact with Hsp70 either via the J domain alone or in conjunction with other, non-conserved, domains (Wall et al 1995). It would appear that the presence of a J domain in all DnaJ proteins even in the absence of a G/F domain will be sufficient to mediate some form of Hsp70 regulation.

The specificity of the J domain/Hsp70 interaction

The question remains as to whether all J domains are functionally equivalent in their interaction with different Hsp70 proteins. For example, yeast Ydj1p interacts specifically with Hsp70 proteins of the SSA but not SSB subfamily (Cyr and Douglas 1994). In addition, Schlenstedt et al (1995) demonstrated that a J domain from one endoplasmic reticulum (ER) DnaJ-like protein was interchangeable for the J domain from another ER DnaJ homologue, but not from non-ER DnaJ proteins. It has been proposed that this specificity could be determined by the amino acids on the outside of the coiled coil of the J domain (Schlenstedt et al 1995; Pellecchia et al 1996). However, given the strong primary sequence homology between all known J domains, it seems likely that the essential components of the J domain structure are conserved with respect to function. If non-conserved residues on the outside of the hydrophobic core do confer some specificity in this interaction, it is likely to result in subtle differences in affinity for various Hsp70 sub-types, whereas the basic mechanism of the J/Hsp70 interaction is universal.

J domain binding sites in Hsp70

The region(s) of Hsp70 that interacts with the J domain are still under investigation, but recent evidence suggests that more than one domain is involved. Deletion of the C-terminal four amino acids of Hsp70 (EEVD) inhibits Hsp40(Hdj1) catalyzed ATP hydrolysis (Freeman et al 1995). The EEVD motif is distal to the Hsp70 peptide binding domain and is conserved in all known Hsp70 proteins. In another study, a single amino acid change in the N-terminal ATPase domain was found to suppress a J domain mutation. The mutation in the J domain was aspartic acid to asparagine of the HPD motif, which inhibited DnaJ stimulated ATP hydrolysis by Hsp70(DnaK). This phenotype was suppressed by an arginine to histidine change in Hsp70(DnaK) at position 167 (W. Suh and C. Gross, personal communication). This position is in the N-terminal domain on the underside of the ATP binding pocket. Together, these data may reflect the interaction of DnaJ with two domains of Hsp70; alternatively, interaction of the J domain with one region may have important consequences for other intramolecular Hsp70 interactions.

Type I DnaJ proteins have zinc fingers

E. coli DnaJ and several of its eukaryotic homologues (see Table) contain a cysteine rich region that has the motif CysXXCys repeated four times. Since similar repeats are found in zinc finger DNA binding proteins, it had been suggested that the DnaJ cys rich domain has zinc dependent tertiary structure. This has now been proven correct by direct studies using purified DnaJ protein (Banecki et al 1996; Szabo et al 1996). Each molecule of *E. coli* DnaJ does indeed bind two Zinc ions that are tetrahedrally coordinated by the sulfur atoms of four cysteine residues. The cys rich domain, therefore, is in fact two zinc fingers that may form a pocket with a hydrophobic core. The functional significance of this structural organization was probed after depletion of Zn^{2+} from wild-type DnaJ, expression of DnaJ mutants lacking this region and expression of the zinc finger domains in the absence of the J and G/F domains (Banecki et al 1996; Szabo et al 1996). These combined studies suggest that the DnaJ zinc fingers are part of a peptide binding domain that is in part responsible for the chaperone function of DnaJ. For example, the zinc finger domain alone can prevent the aggregation of denatured rhodanese, although only full length DnaJ can facilitate complete protein refolding. The zinc fingers do not, however, comprise the entire peptide binding region of DnaJ, since binding of σ^{32} is not compromised in a DnaJ mutant lacking this region. On the other hand, binding of two other DnaJ substrates, RepA and lambdaP is reduced by approximately 2-fold in the same mutant (Banecki et al 1996).

The emerging picture, therefore, is that type I DnaJ proteins comprise three functionally distinct regions: the J and G/F domains, that regulate Hsp70; the zinc finger peptide binding domain and the C-terminal domain, which has yet to undergo thorough characterization, but appears to be a more specialized region for binding some DnaJ substrates. However, the eukaryotic DnaJ family is more diversified than the Hsp70 family. Of the 15 yeast genes that have a J domain only 4 have zinc fingers.

ROLE OF DNAJ-LIKE PROTEINS IN PROTEIN FOLDING

Several studies have now demonstrated a role for some DnaJ-like proteins together with Hsp70 in protein folding; a reaction requiring multiple rounds of interaction between the chaperones and the polypeptide (Hendrick et al 1993; Schroder et al 1993; Szabo et al 1994; Freeman and Morimoto 1996; Levy et al 1995; Schumacher et al 1996). Although the precise mechanism by which Hsp70 and DnaJ-like proteins function in the folding process is unknown, the role of DnaJ-like proteins can be resolved into at least two components; the direct binding to substrate polypeptides and the ability to regulate Hsp70.

It appears that DnaJ-like proteins cannot facilitate protein folding in the absence of Hsp70 (Szabo et al 1994; Levy et al 1995). However, there is evidence that DnaJ (Langer et al 1992), Ydj1p (Cyr 1995) and Mdj1p (Prip-Buus et al 1996) can prevent protein aggregation by themselves. On the other hand, Hsp40(Hdj1) cannot prevent aggregation by itself (Freeman and Morimoto 1996; Minami et al 1996). This suggests that a general role in preventing protein aggregation may be limited to type I DnaJ proteins (i.e. those that possess a zinc binding domain), whereas type II and III DnaJ proteins may facilitate protein folding either by specific substrate presentation and/or by the regulation of Hsp70 substrate binding.

Langer et al (1992) proposed that *E. coli* DnaJ targeted translating polypeptides for subsequent interaction with Hsp70, and that this ternary complex could stabilize a polypeptide against aggregation with other nascent chains. The folding event itself was left to the GroEL chaperone machine. Subsequent studies in eukaryotes suggested this pathway was conserved (Frydman et al 1994). However, eukaryotic cytosolic chaperonins appear to be more selective than GroEL with respect to substrate implying that chaperones other than chaperonins are responsible for the majority of protein folding events in the eukaryotic cytosol and ER (see Hartl 1996 for review). Since DnaJ-like proteins and Hsp70 can assist protein folding independently of chaperonins, it seems likely that this chaperone pair will be involved the biogenesis of many proteins perhaps with the assistance of other molecular chaperones.

Eukaryotic DnaJ proteins interact with Hsp90

Recent biochemical and genetic studies have identified DnaJ-like proteins as potential components of the Hsp90 chaperone machine. In yeast, a synthetic lethal phenotype results when mutations in the genes encoding Hsp90 and Ydj1p are combined (Kimura et al 1995), suggesting that they interact with each other or participate in the same processes. Also, low levels of several rabbit DnaJ-like proteins co-purify with Hsp90, as noted by Schumacher et al (1996), although it remains unclear whether this interaction is direct or indirect. Further evidence that DnaJ-like proteins function in association with Hsp90 was derived from the findings that mutations in yeast *ydj*1 affected Hsp90-mediated signal transduction pathways. In these studies, the activities of steroid hormone receptors and the protein tyrosine kinase $p60^{v\text{-}src}$ were analyzed in *ydj*1 mutant yeast strains. In both cases, there were pleiotropic phenotypes that were allele dependent. In the case of steroid hormone receptors, a glycine to aspartate mutation at position 315 of *ydj*1 led to hormone-independent transactivation by glucocorticoid and estrogen receptors (Kimura et al 1995).

However, in a different *ydj*1 mutant which has several different point mutations (*ydj*1 –151) there was a strong decrease in hormone dependent transactivation by the androgen and glucocorticoid receptors (Caplan et al 1995). These allele dependent phenotypes probably reflect different roles of Ydj1p in the activation process. The different roles for Ydj1p may also reflect its function in different cellular compartments, including the nucleus. Tang et al (1997) recently demonstrated that overexpression of HDJ2 (a human YDJ1 homologue) affected the intranuclear distribution of mutant glucocorticoid receptors in COS cells. Eukaryotic DnaJ proteins may therefore function in both the cytosol and the nucleus in regulating the activation of steroid hormone receptors.

Allele dependent phenotypes were also observed when $p60^{v\text{-}src}$ was expressed in *ydj*1 mutant strains. Expression of $p60^{v\text{-}src}$ in wild-type yeast manifests in a lethal phenotype. In the ydj1–151, ydj1–G313D and ydj1–39 (which has two J-domain mutations) mutants, $p60^{v\text{-}src}$ was inactive and the lethal phenotype was suppressed (Kimura et al 1995; Dey et al 1996). In a ydj1 null mutant, however, there was normal $p60^{v\text{-}src}$ expression and the cells died (Dey et al 1996). These data indicate that Ydj1p is not essential for $p60^{v\text{-}src}$ activity in yeast, but that the protein facilitates its proper folding and activation. In the mutants where the lethal phenotype was suppressed, the different alleles must be exerting a negative effect on the folding process. This has been characterized to some extent, since the levels of $p60^{v\text{-}src}$ are decreased in the ydj1–39 mutant, indicating that Ydj1p affects some aspect of $p60^{v\text{-}src}$ expression and/or stability. In the ydj1–151 mutant, there were normal $p60^{v\text{-}src}$ protein levels, although the protein was completely inactive. Furthermore, both Hsp90 and Hsp70 appear to form a more stable complex with $p60^{v\text{-}src}$ in the ydj1–151 mutant compared to the wild-type. These data suggest that Ydj1p has multiple roles in both $p60^{v\text{-}src}$ folding and in steroid receptor activation.

Role of DnaJ-like proteins in protein degradation

E. coli DnaJ is an important mediator of both general proteolysis (Straus et al 1988; Sherman and Goldberg 1992) and the targeted degradation of certain substrates, for example, σ^{32} (Bukau 1993). Recently, it has been shown that eukaryotic DnaJ-like proteins are also involved in the regulation of protein degradation. The mitochondrial protease PIM1 requires molecular chaperones, including the mitochondrial DnaJ protein Mdj1p, to keep misfolded proteins in a soluble state for degradation (Wagner et al 1994). Ydj1p also appears to be involved in ubiquitin-dependent proteolysis of certain proteins (Lee et al 1996) and the degradation of Cln3 (Yaglom et al 1996). Therefore, DnaJ-like proteins may participate in proteolysis either by 'tagging' certain substrates for degradation (e.g. σ^{32}) or by assisting the unfolding of folded proteins to allow degradation by the proteolytic machinery.

DnaJ proteins in exocytosis and endocytosis

In bacteria, DnaJ and Hsp70(DnaK) are known to participate in secretion, particularly when there are mutations or deletions of other secretory proteins (Wild et al 1992). Indeed, one of the first defined functions for eukaryotic DnaJ-like proteins was their involvement in protein translocation (Caplan et al 1992; Feldheim et al 1992). In many ways this involvement can be viewed as an extension of the chaperone activity of DnaJ-like proteins; however, recent investigations of two type III DnaJ proteins, cysteine string proteins (csp) and auxilin, suggest that DnaJ-like proteins may play a more specialized role in exocytosis and endocytosis. csp were initially identified in Drosophila as a brain and retina specific gene (Zinsmaier et al 1990) and subsequently Torpedo analogues were discovered in a screen for components of voltage-gated calcium channels (Gundersen and Umbach 1992). Detailed analysis showed that csp appeared to be localized on the cytoplasmic face of synaptic vesicles anchored by acyl attachments to their 'cysteine string' (Mastrogiacomo et al 1994b) and mutagenesis of Drosophila csp resulted in a block of synaptic transmission and neurodegeneration (Zinsmaier et al 1994). Therefore, the evidence seemed to strongly suggest that csp were, in some way, mediating regulated synaptic vesicle fusion. However, csp expression does not appear to be restricted to neuronal cells, as they are also expressed in other secretory tissues (Braun and Scheller 1995; Chamberlain and Burgoyne 1996) and are situated on bovine chromaffin granule cells (Chamberlain et al 1996) in a similar way to their location on synaptic vesicles. Therefore, it appears that csp have a wider role in regulated exocytosis than first thought, although it is possible that a family of csp exists in which some isoforms are synapse specific. The mechanism of their action in regulated exocytosis, however, still remains to be defined. Given that csp can regulate Hsp70 in a similar way to other DnaJ family members (Braun et al 1996; Chamberlain and Burgoyne 1997), it is likely that Hsp70 will also cooperate in this process, though at the moment it would appear that the exact mechanism will differ from a classical chaperone role in secretion.

The first known function of the constitutive mammalian Hsp70, Hsc70, was as the enzyme that catalyzed the removal of clathrin coats from coated vesicles, hence the clathrin uncoating ATPase (Ungewickwell 1985; Chappell et al 1986). As we learned more of the role of DnaJ-like proteins as regulators of Hsp70, it became increasingly

likely that a DnaJ would cooperate with Hsc70 in this process. Indeed, it appears that a specialized DnaJ protein, auxilin, forms part of the clathrin basket and facilitates the correct positioning of Hsc70 on the assembled triskelion and assists uncoating (Ungewickell et al 1995). Furthermore, if another DnaJ-like protein (HSJ1) is added to an uncoating reaction, containing Hsc70 and intact coated vesicles, it interferes with the action of this specialized uncoating DnaJ protein, resulting in the stabilization of non-productive Hsc70: clathrin complexes (Cheetham et al 1996). Studies by King et al (1997) have also shown that other soluble exogenous DnaJ proteins, Hsp40(Hdj1) and Ydj1p, also inhibit uncoating. The evolution of auxilin and csp (both type III proteins) illustrate how, by exploiting the J domain, Hsp70 proteins can be targeted to particular cellular locales to do a required function, in this case to fulfill specialized requirements in both exocytosis and endocytosis.

Emerging biology of DnaJ proteins

Despite its name, *E. coli* DnaJ itself does not bind specifically to DNA. However, several other DnaJ-like proteins do have this property, including: CbpA (Ueguchi et al 1994); Zuotin, a yeast Z-DNA and tRNA binding protein (Zhang et al 1992; Wilhelm et al 1994); and the polyoma and papova viral T antigens. The similarity between the N-terminus of T antigens and the J domain was first noted several years ago (Cheetham et al 1992; Kelley and Landry 1994), and recent studies indicate that this similarity has a functional significance. Kelley and Georgeopoulos (1997) demonstrated that the T antigen first exon can functionally replace the J domain of *E. coli* DnaJ in a chimeric protein. Perhaps more significantly, the reciprocal experiment has been performed, replacing the first exon of SV40 T antigen with the J domain of HSJ1 or HDJ2, leading to a chimeric protein that functions essentially as T antigen (Campbell et al 1997). Furthermore, mutagenesis of residues in the HPD motif compromise many of the cellular functions of T antigen, including the degradation of an Rb-related regulatory proteins p130 and p107 (Campbell et al 1997; Stubdal et al 1997). Results from direct biochemical studies also indicate that this region of T antigen is a functional J domain, since it can stimulate the ATPase activity of Hsp70 and catalyze dissociation of Hsp70: CMLA complexes (Srinivasan et al 1997). Although the precise role of the J domain in T antigen's varied cellular functions still remain to be determined, given the known functions of the J domain it is likely that Hsp70 also will participate in these, perhaps involving the known interaction between Hsp70 and Rb or p53.

The T antigen is not the only protein that contains a J domain to be implicated in viral infection, the cell cycle and immortality control. Influenza virus avoids the translational inhibitory affects of a host defense mechanism, the interferon induced RNA-dependent protein kinase (PKR), by activating a cellular inhibitory protein p58IPK. p58IPK is a DnaJ-like protein (type III) and is also a member of the tetratricopeptide (TPR) family of proteins (Lee et al 1994). Overexpression of p58IPK in NIH 3T3 cells transforms their phenotype and makes them tumorigenic (Barber et al 1995), such that p58IPK can mediate malignant transformation. This transformation may be mediated by a down-regulation of PKR and although the J domain does not appear to be required for PKR binding (Polyak et al 1996), the activation of p58IPK itself appears to be regulated by the binding of another DnaJ protein, Hsp40(Hdj1) (Melville et al 1997). Therefore, influenza virus may regulate PKR activity, and hence mRNA translation, via a recruitment of the cellular stress response and manipulation of p58IPK.

A mouse type III DnaJ protein with homology to Zuotin and c-Myb (MIDA1) binds the differentiation and growth regulatory protein Id (Shoji et al 1995). Inhibition of MIDA1 strongly interferes with cell growth, suggesting that MIDA1 can also regulate cell growth and act upon the cell cycle. In contrast, the Drosophila protein Tid56 was identified as derived from part of a genetic locus implicated in tumor suppression (Kurzik-Dumke et al 1995).

While most of the processes discussed above implicate DnaJ proteins as co-chaperones of larger Hsp70 or Hsp90 complexes, there is new evidence for independent functions. One of these is as a protein di-sulphide isomerase as discussed by de Crouy-Chanel et al (1995). This enzymatic property of DnaJ depends on functional cysteine residues, and may depend on the cysteine repeated regions found in central portion of type I DnaJ proteins.

CONCLUSION

Many different forms of DnaJ-like proteins have evolved with diverse cellular localization and functions. Examination of data emerging from the many genome mapping projects reveals large numbers of expressed sequence tags (ESTs) that possess a J domain, and suggests that the DnaJ family may be the largest of all the chaperone families (Pahl et al 1997). Only when the mapping is finished will we know the true extent of DnaJ family diversity, but from what we know already there are potentially very many specialized forms that will confer specificity to chaperone function.

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